

Original Article

PIPKI-PIP2 promotes cell migration by recruiting Smurf1 to the membrane and increasing its activity

Yuxin Chen^{1,2,†}, Xiao Tan^{1,†}, Meiling Lu¹, Yunfei Chen¹, Weijuan Pan³, Rong Wei^{1,*}, and Yingcong Wang^{1,3,*}

¹Department of Hematology, Tongji University Cancer Center, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China, ²School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing 211198, China, and ³Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai 200241, China

[†]These authors contributed equally to this work.

*Correspondence address. E-mail: wangyingcong999@126.com (Y.W.); wei_rong001@163.com (R.W.)

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Abstract

Smurf1 is a member of the Nedd4 family of E3 ubiquitin ligases. Numerous lines of evidence indicate that the membrane localization of Smurf1 is essential for its activity. However, the underlying mechanisms that regulate the membrane localization of Smurf1 remain unclear. Type I phosphatidylinositol phosphate kinase (PIPKI) is a phosphatidylinositol kinase that generates phosphatidylinositol 4,5-bisphosphate (PIP2), which is located in the plasma membrane and regulates cellular processes, including ion channel activity and cell migration. In this study, we show that PIP2 and PIPKI regulate the membrane translocation of Smurf1. Importantly, the recruitment of Smurf1 to the cell membrane through the association of its C2 domain with PIPKI-produced PIP2 is essential for Smurf1-mediated E3 ligase activity and cell migration. Therefore, we identify a PIPKI-PIP2-Smurf1 signaling axis that regulates cell migration.

Key words PIP2, PIPKI, Smurf1, E3 ligase activity, cell migration

Introduction

Smurf1 is homologous to the E6AP carboxyl terminus (HECT) E3 ubiquitin ligase and plays critical roles in cell polarity, cell migration, and development via targeting various substrates, such as Smad, RhoA, and Talin [1–5]. Smurf1 negatively regulates osteoblast activity and the BMP response by ubiquitinating and degrading MEKK2, thereby preventing age-dependent bone mass accumulation through JNK signaling activation [6,7]. Smurf1 contains a C2 domain, a WW domain, and a HECT domain [8,9]. The C2 domain of Smurf1 is involved in direct binding to some substrates, such as RhoA and hPEM-2 [5,10,11]. In addition, the C2 domain of Smurf1 is required for membrane localization [2,12]. The membrane localization of Smurf1 is essential for its E3 ubiquitin ligase activity and cell migration [2,13]. However, the underlying mechanisms that regulate the membrane localization of Smurf1 and its functions in cell migration remain elusive.

Phosphatidylinositol 4,5-bisphosphate (PIP2) has been reported to be involved in diverse cellular processes, including focal

adhesion formation, proliferation, and migration, through the recruitment and/or regulation of cytosolic proteins [14–16]. Type I phosphatidylinositol phosphate kinase (PIPKI) is a member of the phosphatidylinositol kinase family that generates PIP2 through the phosphorylation of phosphatidylinositol 4-phosphate (PI4P) at the D-5 position of the inositol ring [14,17,18]. PIPKI plays an important role in the polarity and migration of both adherent cells and leukocytes [19]. The binding of Talin re-localizes PIPKIγ2 to focal adhesions and regulates localized PIP2 levels and cell migration [17,20]. Our recent results revealed that PIPKI is a substrate of the E3 ubiquitin ligase Smurf1 and that the PKA-Smurf1-PIPKI signaling axis plays an important role in lung cancer cell growth [9]. Furthermore, we were interested in whether PIPKI regulates Smurf1-mediated activity and function.

In this study, we revealed that binding of PIP2 to the C2 domain of Smurf1 is essential for Smurf1 membrane recruitment. PIP2 and PIPKI are required for the E3 ligase activity and cell migration of Smurf1.

Materials and Methods

Cell culture and transfection

HEK293T, H1299 and A549 cells were commercially obtained from the ATCC (Manassas, USA). HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, USA) at 37°C under 5% CO₂. H1299 and A549 cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% FBS. Transfections were performed using the calcium phosphate-DNA co-precipitation method in HEK293T cells. Transfections of H1299 cells were performed via Lipofectamine 2000 (Invitrogen) following the manufacturer's manual.

Antibodies and plasmids

Antibodies against Flag (F7425; Sigma, St Louis, USA), GFP (Santa Cruz Biotech, Santa Cruz, USA), PIP3I (2945-1; Epitomics, Guangzhou, China), and Actin (Abmart, Wuhan, China) were obtained from commercial sources. 4,6-Diamidino-2-phenylindole (DAPI) and MG132 were purchased from Sigma. The expression plasmids used for PIP3I and Smurf1 were described in our previous report [9].

siRNA-mediated knockdown

Cells were transfected with the siRNA oligonucleotides using Lipofectamine. The cells were harvested 72 h post-transfection and analyzed by western blot analysis. The siRNA oligonucleotide sequences used were as follows: control siRNA, 5'-UUCUCCGAAC GUGUCACGUTT-3'; siSmurf#1: 5'-AACCUUGCAAAGAAAGA CUUC-3'; siSmurf#2: 5'-GCAGUUAUCCGGAACAUUUA-3'; siPIP3I#1: 5'-GGACCUGGACUUCAGCAG-3'; and siPIP3I#2: 5'-GAGC GACACAUAAUUUCUA-3'.

Western blot analysis

Western blot analysis was performed as previously described [9]. Briefly, 10 µg of protein was separated by 10% or 12% SDS-PAGE and transferred onto NC membranes (Millipore, Billerica, USA). The membranes were blocked with 5% non-fat milk. The membranes were subsequently incubated with primary antibodies at concentrations recommended by the manufacturer's protocol. After secondary antibody incubation and visualization, the protein bands were detected using the Odyssey system (LI-COR Biosciences-Biotechnology, Lincoln, USA).

Immunofluorescence staining

Cells grown on glass coverslips were fixed with 4% paraformaldehyde (PFA) for 15 min at room temperature (RT), followed by permeabilization with 0.1% Triton X-100 for 10 min. After the samples were blocked with 5% bovine serum albumin (BSA) for 1 h, they were incubated overnight at 4°C with primary antibodies diluted in blocking buffer. Following three washes with PBS, fluorophore-conjugated secondary antibodies (Alexa Fluor 488/594, ab150077; Abcam, Cambridge, UK) were applied for 1 h at RT in the dark. The nuclei were counterstained with DAPI (1 µg/mL, 5 min), and coverslips were mounted with antifade mounting medium. Fluorescence images were acquired and analyzed using a Leica SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany), with appropriate controls (no primary antibody, isotype control) included to ensure specificity.

Cell polarity

To examine cell polarity, transfected H1299 cells were cultured on gelatin-coated coverslips until confluency. The scratch wounds were created using 200-µL pipette tips and washed with PBS to remove the detached cells. The remaining cells were allowed to migrate for 6 h before being fixed with 4% PFA. The cells were permeabilized with 0.1% Triton-X before being blocked with 1% BSA, followed by an overnight incubation with a Golgi antibody (1837-1; Epitomics) at 4°C. The cells were visualized with secondary antibodies conjugated with Texas Red (Thermo Fisher Scientific, Waltham, USA). Images were analyzed using a Leica SP5 confocal microscope (Leica Microsystems).

Lipid binding assay

PIP strips (Echelon Biosciences, Salt Lake, USA) were immersed in blocking buffer (PBST, 0.1% Tween-20, 3% BSA) at room RT for 1 h. The strips were incubated for 2 h at RT with the indicated GST fusion protein (0.5 µg/mL) in the presence of an anti-GST antibody (Beyotime, Shanghai, China). The blots were then washed in PBST three times for 10 min each and incubated with secondary antibody (926-32212, 926-32213; LI-COR Biosciences) in blocking buffer for 1 h. The immunoblots were analyzed using the Odyssey system (LI-COR Biosciences).

Cell migration assay

Cell migration assays were performed as described previously [21]. Briefly, Transwell migration chambers (8 µm pore size; Millipore) were used according to the manufacturer's instructions. In brief, the bottoms of the chambers were filled with 500 µL of RPMI 1640 containing 10% FBS. The H1299 cells were loaded into the upper wells and allowed to migrate for 10 h. The migrated cells were fixed and stained with 1% crystal violet. Images were captured via an inverted microscope (Olympus, Tokyo, Japan), and the number of cells in five random fields on the underside of the filter was counted manually. Three independent experiments were performed.

For the wound healing assay, transfected H1299 cells were seeded and scratched with 200 µL micropipette tips when the cells had grown to near confluency, and the cells were treated with mitomycin C (10 mg/mL; Sigma) to inhibit cell proliferation. This time point was designated as 0 h. Three fixed fields of view per scratch were imaged under an inverted microscope. The plate was returned to the incubator for continued culture, and the same fields were re-imaged at 24 h. The scratch width at each time point was measured using ImageJ software.

In vivo lung metastasis assay in nude mice

In vivo lung metastasis assay was performed using nude mice (6-week-old male BALB/c nude mice, *n* = 6 per group; Vital River Laboratories, Beijing, China) injected with A549 cells (1 × 10⁶ cells in 100 µL of PBS) through the tail vein. The experimental groups included (1) the control group (empty vector-transfected A549 cells), (2) the PIP3I knockout group, (3) the Smurf1-overexpressing group, and (4) the Smurf1-overexpressing cells with PIP3I knockout group. After 6 weeks, the lungs were harvested, and metastatic nodules were quantified. Lung tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned for hematoxylin and eosin (H&E) staining to confirm metastatic lesions. All animal studies were approved by the Institutional Review Board of Shanghai Tenth People's Hospital (ID: SYXK 2011-0112).

Hematoxylin and eosin (H&E)

Formalin-fixed, paraffin-embedded tissue sections (4 ~ 5 μ m) were deparaffinized in xylene and rehydrated through a graded ethanol series. Subsequently, the sections were stained with Harris hematoxylin (Solarbio, Beijing, China), differentiated in 1% acid alcohol, and blued in ammonia water. Counterstaining was performed with eosin (Beyotime), followed by dehydration through ethanol, clearing in xylene, and mounting with a synthetic resin.

Statistical analysis

GraphPad Prism 9 software was used for statistical analysis. All experiments were performed independently at least twice with similar results. Data are shown as the mean $\pm$ SD. Statistical significance was evaluated using the unpaired two-tailed *t* test for comparisons of two mean values. One-way ANOVA was used for comparisons of multiple mean values, and two-way ANOVA was used for comparisons of multiple mean values under different conditions. *P* < 0.05 was considered to be statistically significant.

Results

Binding of PIP2 recruits Smurf1 to the plasma membrane

PIPKI is a lipid kinase required for the production of PI(4,5)P2 in cells [22,23]. Accumulating evidence shows that PIPKI regulates the function of its binding partners by increasing the level of localized PIP2 [19,23,24]. However, whether the binding of phospholipids is required for the membrane localization of Smurf1 is still unclear. We reported that PIPKI binds to the C2 domain of Smurf1 [9], which prompted us to test whether PIPKI can affect Smurf1 activity and/or subcellular localization by promoting the binding of the Smurf1 C2 domain to PI(4,5)P2, which consequently recruits Smurf1 to the plasma membrane. First, we examined the ability of the C2 domain to bind to various phospholipids via a lipid strip assay. As shown in Figure 1A, the wild-type C2 domain shows strong affinity for cardiolin, PI4P, PI(4,5)P2, and PI(3,4,5)P3 and weaker affinity for PA. A previous report revealed that mutation of K28 and K85 in Smurf1 abolished the plasma membrane localization of Smurf1 [5,9]. Thus, we tested whether this mutant is also defective in PIP2 binding. Consistent with a previous report [10], mutation of K28 and K85 in Smurf1 dramatically abolished the ability of Smurf1 to bind to the plasma membrane (Figure 1B). Moreover, the K28/85A mutant dramatically reduced its ability to bind to PI(4,5)P2 and PI(3,4,5)P3 but still efficiently bound to PI4P (Figure 1A). Under basal conditions, the level of PI(4,5)P2 is much higher than that of PI(3,4,5)P3 at the plasma membrane. Combined with their membrane localization, we hypothesized that the binding of PI(4,5)P2 via the C2 domain is sufficient for the recruitment of Smurf1 to the plasma membrane.

To test this hypothesis, Smurf1 was coexpressed with the yeast phospholipid phosphatase SopB, which dephosphorylates 4'-PIs and 5'-PIs *in vivo* and thus decreases the level of PI(4,5)P2 in cells. As shown in Figure 1C, coexpression of active SopB (SopB WT), but not activity-dead SopB (SopB MT), impaired the membrane localization of Smurf1. Furthermore, we constructed the plasmids PM-GFP-INPP5 and GFP-INPP5. PM-GFP-INPP5, which contains a membrane localization signal (PM) enabling specific plasma membrane targeting, effectively hydrolyzes PIP2 at the membrane. GFP-INPP5, which fails to localize to the plasma membrane,

impairs PIP2 hydrolysis. We co-transfected the plasmids PM-GFP-INPP5 and GFP-INPP5 with Smurf1 and examined the localization of Smurf1. The results revealed that cotransfection with PM-GFP-INPP5E resulted in the cytoplasmic redistribution of Smurf1, whereas cotransfection with GFP-INPP5 maintained Smurf1 membrane localization (Figure 1D).

To further confirm this result, we substituted the C2 domain of Smurf1 with the PH domain of PLC-D, which has been shown to have high affinity for PIP2. As expected, deletion of the C2 domain completely impaired the membrane localization of Smurf1. Replacement of C2 by the PH domain (Smurf1 PH) also relocalized Smurf1 to the plasma membrane (Figure 1E). Together, we concluded that the binding of PI(4,5)P2 via the C2 domain is essential for the localization of Smurf1 to the plasma membrane.

PIPKI is required for the membrane localization of Smurf1

Since PI(4,5)P2 is required for the membrane localization of Smurf1, PIPKI is a major lipid kinase required for the production of PI(4,5)P2 in cells; thus, we tested the role of PIPKI in Smurf1 membrane localization. When endogenous *PIPKI* was knocked down using siRNA and co-transfected with Smurf1, the results revealed that PIPKI depletion significantly impaired the membrane localization of Smurf1 (Figure 2A). In parallel experiments, when *PIPKI* was knocked down using siRNA and co-transfected with the classical PH domain plasmid (GFP-PLC-PH), PIPKI deficiency markedly disrupted the membrane localization of GFP-PLC-PH (Figure 2B). These results indicate that PI(4,5)P2 and PIPKI are required for the recruitment of Smurf1 to the plasma membrane.

PIP2 and PIPKI regulate the activity of Smurf1

Degradation of RhoA is important for Smurf1-mediated cell migration [25]. The C2 domain has been shown to be essential for the degradation of RhoA by Smurf1 [10]. Therefore, we investigated whether the binding of PIP2 regulates Smurf1 activity toward RhoA. Consistent with a previous report [13], coexpression of wild-type Smurf1 efficiently degraded RhoA, and deletion of the C2 domain abolished its activity toward RhoA. Interestingly, replacement of the PH domain rescued Smurf1-mediated RhoA degradation, indicating that the PH domain can partially rescue the effect of C2 deletion (Figure 3A). Moreover, Smurf C699A, which is an E3 ubiquitin ligase activity-deficient mutant of Smurf1, fails to degrade RhoA (Figure 3B). To investigate whether PIPKI affects Smurf1-mediated degradation of RhoA, we cotransfected cells with PIPKI and Smurf1 and measured the RhoA protein level. The results showed that PIPKI enhanced Smurf1-mediated RhoA degradation in a dose-dependent manner (Figure 3C). These results suggest that the recruitment of Smurf1 to the plasma membrane by the binding of PIP2 is required for Smurf1-mediated cell migration and RhoA degradation. Smurf1 has been shown to be required for the polarity of epithelial cells [26]. To evaluate cell polarity, we performed Golgi staining. In polarized cells, the Golgi apparatus is confined to a 120° sector at the nuclear anterior pole, which coincides with the migration direction. As shown in Figure 3D, *Smurf1* knockdown impaired lung cancer cell polarity. The expression of the shRNA-resistant Smurf1 WT strain but not the Smurf1 Δ C2 strain rescued the Smurf1 depletion phenotype. Interestingly, the expression of

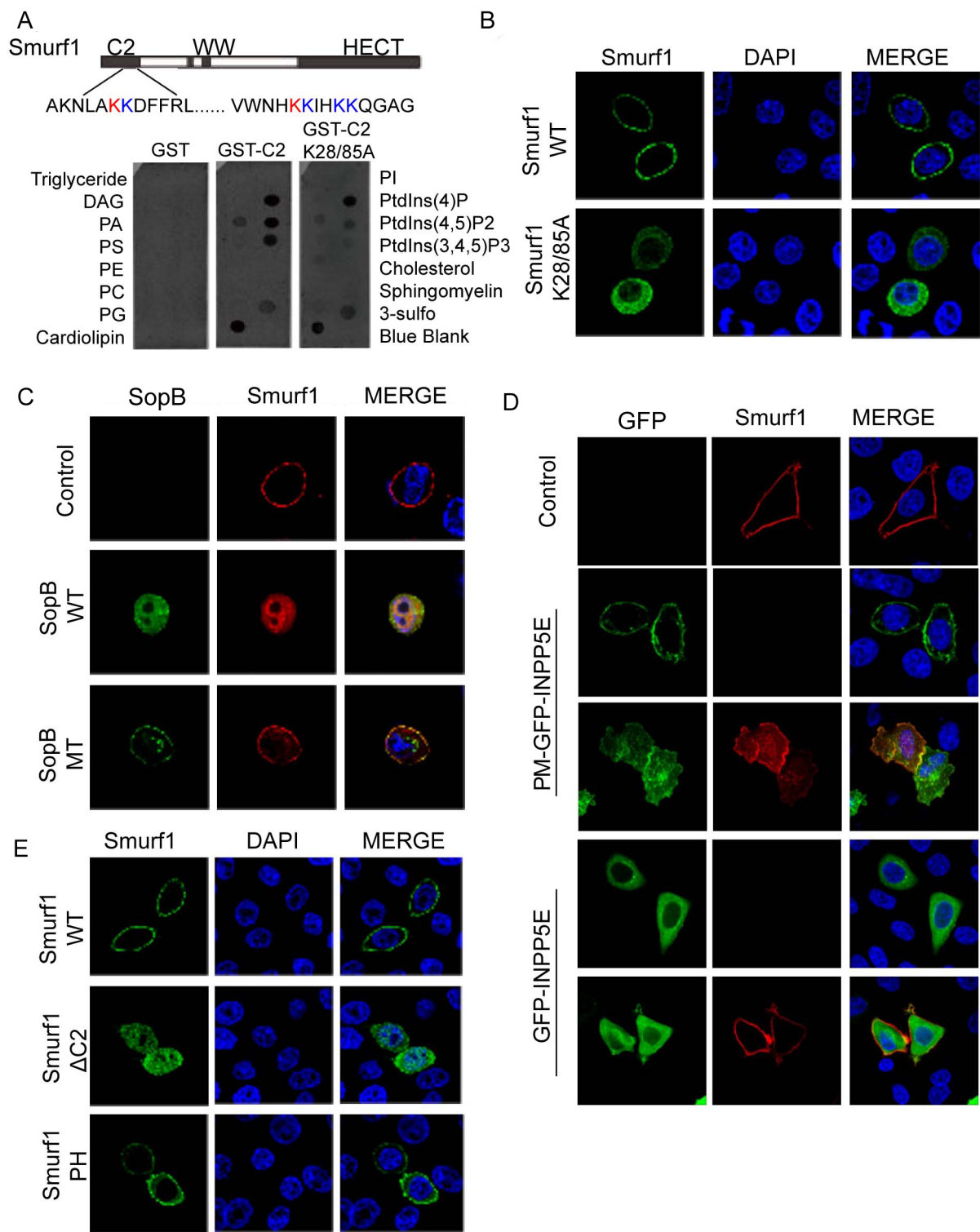


Figure 1. Binding of PIP2 recruits Smurf1 to the plasma membrane (A) Lipid strips reveal GST-fusion protein binding selectivity. The GST-Smurf1 C2 domain, but not the GST domain, interacts with PI4P, PI(3,4,5)P2, PI(3,4,5)P3 and PA. GST-Smurf1 K28/85A shows low affinity for PI(4,5)P2 and PI(3,4,5)P3. (B) The subcellular localization of full-length, double lysine mutants of Smurf1. Flag-tagged wild-type Smurf1 and K28/85A Smurf1 were individually expressed in H1299 cells and stained with an anti-Flag antibody before visualization by confocal microscopy. DAPI, 4,6-diamidino-2-phenylindole. (C,D) Phosphid phosphatases impaired the membrane localization of Smurf1 in H1299 cells. H1299 cells were transfected with plasmids encoding Flag-Smurf1, YFP-SopB WT, YFP-SopB MT, PM-GFP-INPP5E, or GFP-INPP5E and empty vector. The cells were stained with an anti-Flag antibody and DAPI before visualization by confocal microscopy. (E) The subcellular localization of full-length Smurf1, C2 deletion and PH-Smurf1. The indicated plasmids were individually expressed in H1299 cells and stained with an anti-Flag antibody before visualization by confocal microscopy. Magnification, 63x.

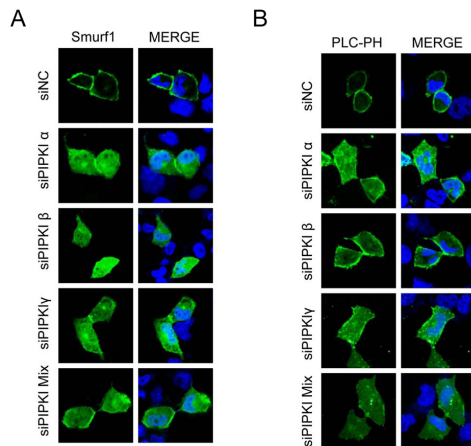


Figure 2. PIPKI is required for the membrane localization of Smurf1 (A) Co-transfection of siRNA targeting endogenous PIPKI with Smurf1 revealed that *PIPKI* knockdown significantly impaired Smurf1 membrane localization. (B) After co-transfection with the canonical PH domain construct (GFP-PLC-PH), PIPKI depletion was also found to markedly disrupt GFP-PLC-PH membrane targeting. The cells were stained with DAPI before visualization by confocal microscopy. Magnification, 63x.

shRNA-resistant Smurf1 PH also rescued the defect in cell polarity caused by Smurf1 depletion (Figure 3D). These results indicate that PIP2 and PIPKI regulate Smurf1 activity via the recruitment of Smurf1 to the plasma membrane.

PIPKI and PIP2 are required for Smurf1-mediated lung cancer cell migration

Smurf1 has been shown to promote the migration of HEK293T cells and breast cancer cells [27]. We tested whether Smurf1 is also required for lung cancer cell migration. As shown in Figure 4A,B, ectopic expression of wild-type Smurf1 markedly increased the migration of H1299 cells. Depletion of the C2 domain or the K28A/K85A mutation failed to promote cell migration. Interestingly, replacement of the C2 domain by the PH domain rescued Smurf1-promoted cell migration, suggesting that the binding of PI(4,5)P₂ to the C2 domain is required for Smurf1-mediated lung cancer cell migration. We also tested whether PIPKI is required for Smurf1-promoted cell migration. As shown in Figure 4C, knockdown of *PIPKI* impaired Smurf1-induced cell migration. These results suggest that the recruitment of Smurf1 to the plasma membrane by the binding of PIP2 and PIPKI is required for Smurf1-mediated cell migration. Furthermore, we conducted an *in vivo* mouse lung

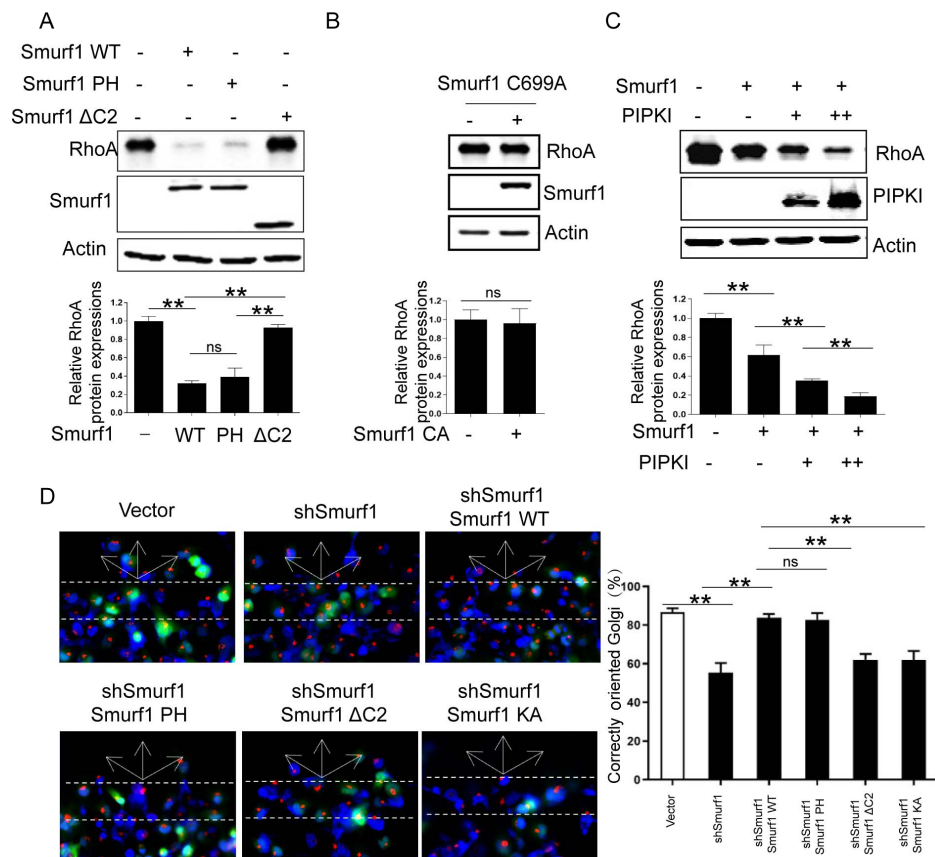


Figure 3. PIPKI and PIP2 regulate the activity of Smurf1 (A) PIP2 regulates Smurf1 activity toward RhoA. FLAG-RhoA was transfected into HEK293T cells in the presence of FLAG-tagged wild-type Smurf1 (WT) and other indicated plasmids. The levels of RhoA were detected using an anti-FLAG antibody. (B) Smurf C699A fails to degrade RhoA. (C) PIPKI increased Smurf1-mediated RhoA degradation. FLAG-RhoA was co-expressed with FLAG-Smurf1, increasing the amount of PIPKI. The degradation of FLAG-RhoA was monitored by immunoblotting. (D) The C2 domain of Smurf1 is required for lung cancer cell polarity during wound healing. H1299 cells stably infected with retrovirus containing shSmurf1 or shVector were transfected with the indicated shRNA-resistant plasmids, and cell polarity during wound healing was measured via Golgi staining. ** $P < 0.01$. ns, not significant. Magnification, 63x.

metastasis experiment. The results showed that the knockout of *PIP1* impaired Smurf1-mediated metastasis. The control group showed metastatic foci averaging 0.3 mm in diameter, whereas Smurf1 overexpression significantly increased the degree of

metastasis (0.6 mm in diameter). Notably, *PIP1* knockout in Smurf1-overexpressing cells reduced metastasis to control levels (0.31 mm diameter), demonstrating that *PIP1* is required for Smurf1-mediated enhancement of metastasis (Figure 4D). In

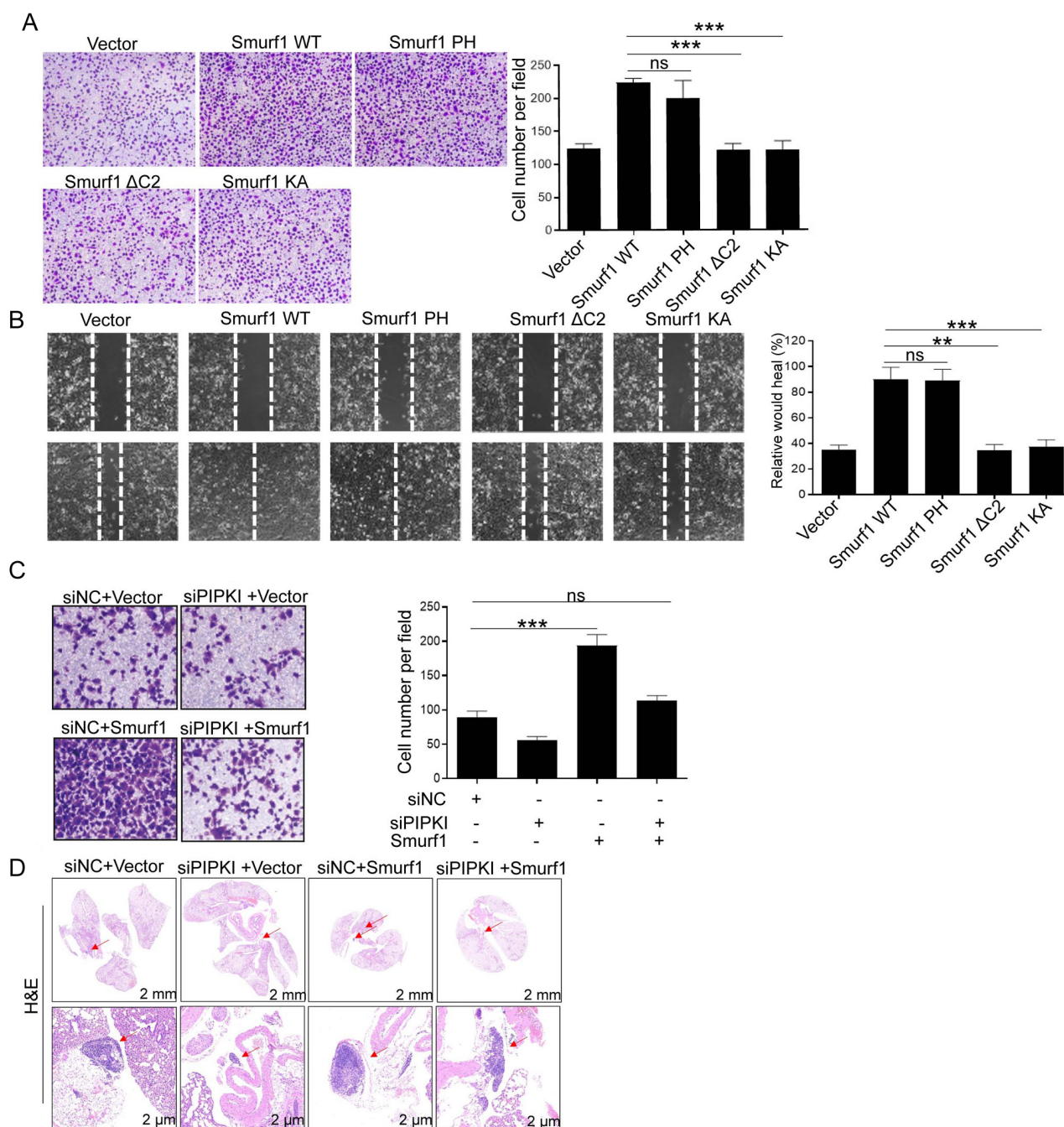


Figure 4. PIPKI and PIP2 are required for Smurf1-mediated lung cancer cell migration (A) The C2 domain is important for Smurf1-mediated lung cancer cell migration. H1299 cells were transfected with the indicated plasmids. Twenty-four hours later, the cells were plated in duplicate in the upper chambers for migration for approximately 12 h. Transwell filters were fixed and stained with crystal violet. The bottoms of the filters were photographed in five independent fields and counted. (B) Binding of PI(4,5)P₂ to the C2 domain is required for Smurf1-mediated lung cancer cell migration. H1299 cells were transfected with the indicated plasmids, and the cell migration ability was examined by wound healing assay. (C) PIPKI is required for Smurf1-promoted cell migration. H1299 cells were transfected with the indicated siRNAs or plasmids, and cell migration ability was measured by transwell assay. (D) PIPKI is essential for Smurf1-mediated lung metastasis *in vivo*. Representative images of lung metastatic nodules (red arrows) in control, Smurf1-overexpressing and *PIP1*-knockdown mice injected with Smurf1-expressing tumor cells. Data are presented as the mean $\pm$ SD (Student's *t* test). * $P < 0.01$, *** $P < 0.001$ ($n = 3$). ns, not significant.

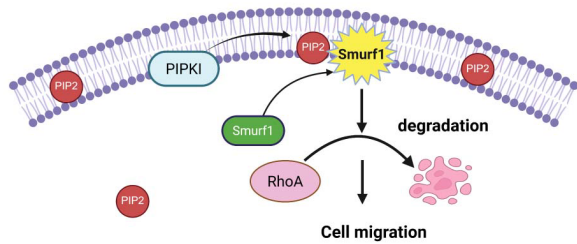


Figure 5. Proposed working model for PIPKI-induced Smurf1 activation in cell migration PIPKI generates PIP2 to recruit and activate Smurf1 at the plasma membrane, promoting cell migration.

summary, PIPKI is essential for Smurf1-mediated lung metastasis.

Discussion

In this study, we found that the binding of PIP2 is essential for the recruitment of Smurf1 to the plasma membrane through the C2 domain. Interestingly, we noted that PIP2 and PIPKI increased Smurf1 activity toward RhoA and Smurf1-mediated cell migration.

Several studies have shown that the C2 domain of Smurf1 is required for its membrane localization and cell migration [8,10]. In addition to Smurf1, other HECT family E3 ubiquitin ligases, including WWP1, WWP2, and Smurf2, also have a C2 domain, which binds to phospholipids [1,28]. However, it is still unclear which phospholipids are required for their membrane localization. It is also unclear whether Smurf1 C2 can bind to phospholipids as well. In our study, we found that the C2 domain of Smurf1 can bind to PI4P, PI(4,5)P2 and PI(3,4,5)P3 *in vitro*. However, a K28/85A mutation, which cannot associate with the cell membrane, significantly reduced the ability of the protein to bind to PI(4,5)P2 and PI(3,4,5)P3. Under basal conditions, the amount of PI(4,5)P2 is much greater than that of PI(3,4,5)P3. Thus, we concluded that the binding of PI(4,5)P2 via the C2 domain is one of the major mechanisms involved in the recruitment of Smurf1 to the cell membrane. Interestingly, degradation of cellular PIP2 significantly reduced the degree of membrane interaction of Smurf1. Although Smurf1 is localized to the membrane, it also degrades nuclear proteins, such as KLF2 [29]. Thus, our study indicated that some signaling pathways that regulate the cellular PI(4,5)P2 level may affect substrate activity toward substrates with different subcellular localizations. Interestingly, our data revealed that substitution of the C2 domain with a PIP2-binding domain can partially rescue the activity of Smurf1 toward RhoA degradation and cell migration, indicating that binding of PIP2 is important for Smurf1 function.

In summary, our study identified a novel mechanism that regulates Smurf1 activity and function (Figure 5). We discovered that PIPKI-PIP2-Smurf1 signaling regulates lung cancer cell migration.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Rotin D, Kumar S. Physiological functions of the HECT family of ubiquitin ligases. *Nat Rev Mol Cell Biol* 2009, 10: 398–409
2. Cao Y, Zhang L. A Smurf1 tale: function and regulation of an ubiquitin ligase in multiple cellular networks. *Cell Mol Life Sci* 2013, 70: 2305–2317
3. Suzuki C, Murakami G, Fukuchi M, Shimanuki T, Shikachi Y, Imamura T, Miyazono K. Smurf1 regulates the inhibitory activity of smad7 by targeting smad7 to the plasma membrane. *J Biol Chem* 2002, 277: 39919–39925
4. Fu L, Cui CP, Zhang X, Zhang L. The functions and regulation of Smurfs in cancers. *Semin Cancer Biol* 2020, 67: 102–116
5. Tian M, Bai C, Lin Q, Lin H, Liu M, Ding F, Wang HR. Binding of RhoA by the C2 domain of E3 ligase Smurf1 is essential for Smurf1-regulated RhoA ubiquitination and cell protrusive activity. *FEBS Lett* 2011, 585: 2199–2204
6. Wan L, Zou W, Gao D, Inuzuka H, Fukushima H, Berg AH, Drapp R, et al. Cdh1 regulates osteoblast function through an APC/C-independent modulation of Smurf1. *Mol Cell* 2011, 44: 721–733
7. Yamashita M, Ying SX, Zhang G, Li C, Cheng SY, Deng C, Zhang YE. Ubiquitin ligase smurf1 controls osteoblast activity and bone homeostasis by targeting MEK2 for degradation. *Cell* 2005, 121: 101–113
8. Koganti P, Levy-Cohen G, Blank M. Smurfs in protein homeostasis, signaling, and cancer. *Front Oncol* 2018, 8: 295
9. Li H, Xiao N, Wang Y, Wang R, Chen Y, Pan W, Liu D, et al. Smurf1 regulates lung cancer cell growth and migration through interaction with and ubiquitination of PIPKI. *Oncogene* 2017, 36: 5668–5680
10. Lu K, Li P, Zhang M, Xing G, Li X, Zhou W, Bartlam M, et al. Pivotal role of the C2 domain of the Smurf1 ubiquitin ligase in substrate selection. *J Biol Chem* 2011, 286: 16861–16870
11. Yamaguchi K, Ohara O, Ando A, Nagase T. Smurf1 directly targets hPEM-2, a GEF for Cdc42, via a novel combination of protein interaction modules in the ubiquitin-proteasome pathway. *Biol Chem* 2008, 389: 405–413
12. Fei C, He X, Xie S, Miao H, Zhou Z, Li L. Smurf1-mediated axin ubiquitination requires Smurf1 C2 domain and is cell cycle-dependent. *J Biol Chem* 2014, 289: 14170–14177
13. Murakami K, Etlinger JD. Role of SMURF1 ubiquitin ligase in BMP receptor trafficking and signaling. *Cell Signal* 2019, 54: 139–149
14. Di Paolo G, Pellegrini L, Letinic K, Cestra G, Zoncu R, Voronov S, Chang S, et al. Recruitment and regulation of phosphatidylinositol phosphate kinase type 1γ by the FERM domain of talin. *Nature* 2002, 420: 85–89
15. Doughman RL, Firestone AJ, Anderson RA. Phosphatidylinositol phosphate kinases put PI4,5P₂ in its place. *J Membrane Biol* 2003, 194: 77–89
16. Ling K, Schill NJ, Wagoner MP, Sun Y, Anderson RA. Movin' on up: the role of PtdIns(4,5)P₂ in cell migration. *Trends Cell Biol* 2006, 16: 276–284
17. Ling K, Doughman RL, Firestone AJ, Bunce MW, Anderson RA. Type 1γ phosphatidylinositol phosphate kinase targets and regulates focal adhesions. *Nature* 2002, 420: 89–93
18. Heck JN, Mellman DL, Ling K, Sun Y, Wagoner MP, Schill NJ, Anderson RA. A conspicuous connection: structure defines function for the phosphatidylinositol-phosphate kinase family. *Crit Rev Biochem Mol Biol* 2007, 42: 15–39
19. Xu W, Wang P, Petri B, Zhang Y, Tang W, Sun L, Kress H, et al. Integrin-

- induced PIP5K1C kinase polarization regulates neutrophil polarization, directionality, and *in vivo* infiltration. *Immunity* 2010, 33: 340–350
20. Ling K, Doughman RL, Iyer VV, Firestone AJ, Bairstow SF, Mosher DF, Schaller MD, *et al*. Tyrosine phosphorylation of type I γ phosphatidylinositol phosphate kinase by Src regulates an integrin-talin switch. *J Cell Biol* 2003, 163: 1339–1349
21. Xiao N, Li H, Luo J, Wang R, Chen H, Chen J, Wang P. Ubiquitin-specific protease 4 (USP4) targets TRAF2 and TRAF6 for deubiquitination and inhibits TNF α -induced cancer cell migration. *Biochem J* 2012, 441: 979–987
22. Wenk MR, Pellegrini L, Klenchin VA, Di Paolo G, Chang S, Daniell L, Arioka M, *et al*. PIP kinase I γ is the major PI(4,5)P $_2$ synthesizing enzyme at the synapse. *Neuron* 2001, 32: 79–88
23. Loijens JC, Anderson RA. Type I phosphatidylinositol-4-phosphate 5-kinases are distinct members of this novel lipid kinase family. *J Biol Chem* 1996, 271: 32937–32943
24. Krauss M, Kinuta M, Wenk MR, De Camilli P, Takei K, Haucke V. ARF6 stimulates clathrin/AP-2 recruitment to synaptic membranes by activating phosphatidylinositol phosphate kinase type I γ . *J Cell Biol* 2003, 162: 113–124
25. van Dijk RA, Engels CC, Schaapherder AF, Mulder-Stapel A, Ten Dijke P, Hamming JF, Lindeman JH. Visualizing TGF- β and BMP signaling in human atherosclerosis: a histological evaluation based on Smad activation. *Histol Histopathol* 2012, 27: 387–396
26. Wang HR, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, Thomsen GH, Wrana JL. Regulation of cell polarity and protrusion formation by targeting rhoa for degradation. *Science* 2003, 302: 1775–1779
27. Kwon A, Lee HL, Woo KM, Ryoo HM, Baek JH. SMURF1 plays a role in EGF-induced breast cancer cell migration and invasion. *Molecules Cells* 2013, 36: 548–555
28. Brigi A, Hofmann L, Argüelles C, Sanial M, Holmgren RA, Plessis A. Control of the dynamics and homeostasis of the *Drosophila* Hedgehog receptor patched by two C2-WW-HECT-E3 ubiquitin ligases. *Open Biol* 2015, 5: 150112
29. Xie P, Tang Y, Shen S, Wang Y, Xing G, Yin Y, He F, *et al*. Smurf1 ubiquitin ligase targets Kruppel-like factor KLF2 for ubiquitination and degradation in human lung cancer H1299 cells. *Biochem Biophys Res Commun* 2011, 407: 254–259